Technical Bulletin

EZview™ Red Anti-c-Myc Affinity Gel

E6654

Product Description

The human c-*myc* proto-oncogene is the human cellular homolog of the avian v-*myc* gene found in several leukemogenic retroviruses.[1-3] Increased expression of the cellular oncogene c-*myc* has been described in various human tumors, occurring by several mechanisms, including gene amplification and chromosomal translocation.[3]

EZview™ Red Anti-c-Myc Affinity Gel is a highly visible, red-colored agarose affinity gel, designed for use in immunoprecipitation experiments. The affinity resin contains an affinity-purified polyclonal anti-c-Myc antibody, developed in rabbit, coupled to cyanogen bromide-activated agarose. Anti-c-Myc was developed in rabbit, using a peptide that corresponds to amino acid residues 408-425 of human c-Myc as the immunogen.

EZview™ Red Anti-c-Myc Affinity Gel recognizes the c-Myc tag peptide (EQKLISEEDL), an epitope located within amino acid residues 410-419 of human c-Myc. The c-Myc tag peptide has been widely used as a tag in many expression vectors, to enable the expression of corresponding c-Myc-tagged fusion proteins.[4]

EZview™ Red Anti-c-Myc Affinity Gel is useful for the purification of expressed c-Myc-tagged fusion proteins from bacterial lysates or from transfected mammalian cells. It binds native as well as denatured or reduced forms of c-Myc-tagged proteins. It is reactive with N-terminal or C-terminal c-Myc-tagged proteins. The c-Myc-tagged proteins, bound to the EZview™ Red Anti-c-Myc Affinity Gel, are recovered by centrifugation.

EZview™ Red Anti-c-Myc Affinity Gel functions in the same manner as the standard non-colored Polyclonal Anti-c-Myc Agarose Conjugate (Cat. No. A7470). The red color gives the affinity gel enhanced visibility, to ease downstream manipulations such as repetitive washings, recovery of the affinity resin beads, and recovery of the bound c-Myc-tagged proteins. The enhanced visibility facilitates sample processing. Several theses[5] and dissertations[6-13] have cited use of this E6654 product in their research protocols.

Reagent

EZview™ Red Anti-c-Myc Affinity Gel is supplied as a ~50% slurry suspension in phosphate buffered saline (PBS), containing 50% glycerol and 0.0015% (15 ppm) Kathon® CG/IPCII, as an antimicrobial preservative. The purified antibody is immobilized at 1.0-1.5 mg of antibody per mL of agarose.

Binding capacity: ~0.05 mg/mL of packed resin

Equipment Required but Not Provided

Suggested Cat. Nos. are provided as appropriate.

- Appropriate lysis buffer, such as CelLytic™ M (Cat. No. C2978), CelLytic™ MT (Cat. No. C3228), or RIPA Buffer (Cat. No. R0278)
- Vortex mixer
- Protease Inhibitor Cocktail (such as Cat. Nos. P8340 or P2714)
- Pipette tips (200 µL)
- Pipette tips, wide orifice (200 µL)
- Pipette tips (1,000 µL)
- Pipette (200 µL)
- Pipette (1,000 µL)
- Microcentrifuge tubes (such as Cat. No. T9661)
- 2× Laemmli Sample Buffer (Cat. No. S3401)

Storage/Stability

EZview™ Red Anti-c-Myc Affinity Gel is stable for at least one year when stored at 2-8 °C. Since this product is a slurry containing 50% glycerol, it is considered to be freezer-safe. For maximum stability, it is suggested to store this product at 0 to -20 °C.

Do not freeze without 50% (v/v) glycerol present in the storage buffer.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Procedure

Various immunoprecipitation (IP) procedures are available.[14] The following procedure is a generic method for affinity capture of c-Myc-tagged proteins for protein-protein interaction studies. It may not be appropriate for all situations. The procedure is written for a single sample and is appropriate for most mammalian tissue culture cell lines. It can be scaled for multiple samples as appropriate. The investigator needs to determine optimal incubation times and conditions. Manipulations should be carried out on ice or at 2-8 °C.

The Lysis Buffer will depend on the organism, type of cells, and experimental objectives. RIPA buffer (Cat. No. R0278), CelLytic™ M (Cat. No. C2978), or CelLytic™ MT (Cat. No. C3228) may be used for lysis of most mammalian cells or tissues.

Immunoprecipitation of c-Myc-tagged proteins

1. Carefully mix EZview™ Red Anti-c-Myc Affinity Gel beads until completely and uniformly suspended. Add 50 µL of the 50% slurry into a clean 1.5 mL microcentrifuge tube on ice. To dispense beads, use a wide orifice pipette tip, or cut ~1 mm off the tip of a regular pipette tip to enlarge the opening and allow unrestricted flow of the bead suspension.
2. Equilibrate beads in Lysis Buffer by adding 750 µL of Lysis Buffer to the tube. Vortex. Centrifuge in a microcentrifuge for ~30 seconds at 8,200 × *g* (10,000 rpm in an Eppendorf® 5415C microcentrifuge).
3. Carefully remove the supernatant with micropipette (or carefully aspirate supernatant) and set the tube with the bead pellet on ice. Equilibrate the beads a second time. After removing the supernatant, set the tube with equilibrated red bead pellet on ice.
4. Prepare the cell lysate using ice cold Lysis Buffer. For most mammalian cells, 0.5-5 × 10^7 cells can be easily lysed in 1 mL of the Lysis Buffer. The appropriate protease inhibitor cocktail may be added to the Lysis Buffer, if desired. Transfer the lysate to a 1.5 mL microcentrifuge tube.
5. Immediately centrifuge the lysate for 10 minutes at 8,200 × *g* in a microcentrifuge at 2-8 °C to pellet cell debris.
6. Carefully remove the clear lysate supernatant from Step 5 with a 1 mL micropipette. Transfer into the tube (from Step 2) of equilibrated EZview™ Red Anti-c-Myc Affinity Gel beads. Vortex briefly. Incubate with thorough, gentle mixing for 1 hour at 2-8 °C to allow c-Myc-tagged proteins to bind to the anti-c-Myc antibody on the EZview™ Red Anti-c-Myc Affinity Gel.
7. Centrifuge in a microcentrifuge for 30 seconds at 8,200 × *g*. Set on ice. Aspirate supernatant carefully (or remove with a micropipette). Set the tube with the bead pellet on ice.
8. Wash the bead pellet by adding 750 µL of Lysis Buffer. Vortex briefly. Incubate with thorough, gentle mixing at 2-8 °C for 5 minutes. Centrifuge in a microcentrifuge for 30 seconds at 8,200 × *g*. Aspirate supernatant carefully (or remove with a micropipette). Set the tube with the bead pellet on ice.
9. Repeat washes two more times as in Step 8. After removing the final wash supernatant, the bound protein can be eluted and analyzed as desired.

Analysis of Bound Protein

Elution of the c-Myc-fusion protein with c-Myc peptide

- The c-Myc-tagged protein bound to the resin may be eluted with c-Myc peptide (Cat. No. M2435).
- Add the desired volume of freshly prepared 100 µg/mL c-Myc peptide in RIPA buffer.
- Incubate the affinity gel sample for at least 5 minutes.
- Recover the supernatant after pelleting the affinity gel by centrifugation.

Enzyme assays

Enzyme assays, such as kinase assays, can be performed by adding the assay mixture and substrate directly into the bead sample tube. The bead pellet should first be equilibrated as in Step 2, except using enzyme assay buffer in place of Lysis Buffer.

SDS-PAGE analysis

- To elute the captured protein from the bead pellet for SDS-PAGE analysis, add 50 µL of 2× Laemmli sample buffer.
- Vortex briefly.
- Boil sample for 5 minutes. Vortex. Centrifuge 30 seconds at 8,200 × *g* in microcentrifuge to pellet EZview™ Red Anti-c-Myc Affinity Gel beads.
- Store the sample frozen, if not used immediately.
- Run 10-20 µL of the supernatant on a denaturing SDS-PAGE gel.
- Perform subsequent detection by staining, autoradiography, or immunoblotting, as desired.

Note: For analysis using non-reducing SDS-PAGE, use a sample buffer without reducing agents such as 2-mercaptoethanol or dithiothreitol. Also, this can lower the amount of anti-c-Myc antibody that is released from the affinity resin.

References

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Notice

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Troubleshooting Guide

The enhanced visibility of the EZview™ Red affinity resin beads makes it easy to see if the beads have been accidentally removed during the wash steps. If this happens, simply return the wash supernatant back to the tube, and repeat the centrifugation step to pellet the resin again.

Problem	Possible Cause	Solution
No signal is observed	c-Myc-tagged protein is not present in the sample.	• Verify that the protein of interest contains the c-Myc-tag by immunoblot or dot blot analyses. • Prepare fresh lysates. Avoid using frozen lysates. • Use appropriate protease inhibitors in the lysate or increase their concentrations to prevent degradation of the c-Myc-tagged protein.
	Washes are too stringent.	• Reduce the number of washes • Avoid adding high concentrations of NaCl to the mixture. • Use solutions that contain less or no detergent.
	Incubation times are inadequate.	Increase the incubation times with the affinity resin (from several hours to overnight).
	Interfering substance is present in sample.	• Lysates containing high concentrations of dithiothreitol (DTT), 2-mercaptoethanol, or other reducing agents may destroy antibody function, and must be avoided. • Excessive detergent concentrations may also interfere with the protein binding interactions. Try diluting the lysate with buffer prior to immunoprecipitation.
	Detection system is inadequate.	If Western blotting detection is used: • Check primary and secondary antibodies with proper controls to confirm binding and reactivity in detection system. • Verify that the transfer was adequate by staining the membrane with Ponceau S. • Try fresh detection substrate or try a different detection system.
Background is too high.	Proteins non-specifically bind to the anti-c-Myc polyclonal antibody, the resin beads, or the microcentrifuge tubes.	• Pre-clear lysate with Rabbit IgG-Agarose (Cat. No. A2909) and/or EZview™ Red Protein A Affinity Gel (Cat. No. P6486) to remove non-specific binding proteins. • After suspending beads for the final wash, transfer entire sample to a clean microcentrifuge tube before centrifugation.
	Washes are insufficient.	• Increase the number of washes. • Prolong duration of the washes, incubating each wash for at least 15 minutes. • Increase the salt and/or detergent concentrations in washing solutions. • Centrifuge at lower speed to avoid non-specific trapping of denatured proteins from the lysate during the initial centrifugation of the affinity resin complexes.

Reagent Compatibility Table

Reagent	Effect	Comments
Chaotropic agents (such as urea, guanidine HCl)	Denatures the immobilized anti-c-Myc antibody	• **Do not** use any reagent that contains chaotropic agents, since chaotropic agents denature the anti-c-Myc antibody on the resin and destroy its ability to bind c-Myc-tagged proteins. • If necessary, low concentrations of urea (1 M or less) can be used.
Reducing agents (such as DTT, DTE, 2-mercaptoethanol)	Reduces the disulfide bridges holding the anti-c-Myc antibody chains together	**Do not** use any reagent that contains reducing agents, since reducing agents will reduce the disulfide linkages in the anti-c-Myc antibody on the resin and destroy its ability to bind the c-Myc-tagged proteins.
Sodium dodecyl sulfate (SDS)	Reduces non-specific protein binding to the resin	May be used up to a concentration of 0.1%. **Do not exceed**, since SDS may denature the anti-c-Myc antibody on the resin and destroy its ability to bind C-myc-tagged proteins.
TWEEN® 20	Reduces non-specific protein binding to the resin	May be used up to a concentration of 5%. **Do not exceed**.
TRITON® X-100	Reduces non-specific protein binding to the resin	May be used up to a concentration of 5%. **Do not exceed**.
IGEPAL® CA-630 (NP-40)	Reduces non-specific protein binding to the resin	May be used up to a concentration of 1%. **Do not exceed**.
Digitonin	Reduces non-specific protein binding to the resin	May be used up to a concentration of 0.2%. **Do not exceed**.
Sodium chloride	Reduces non-specific protein binding to the resin by reducing ionic interactions	May be used up to a concentration of 1.0 M. **Do not exceed**.
0.1 M glycine HCl, pH 2.5	Elutes c-Myc-tagged protein from the resin	Do not leave the affinity resin in glycine-HCl for longer than 20 minutes. Longer incubation times will begin to denature the anti-c-Myc antibody.

Related Products

- Anti-c-Myc-Peroxidase Conjugate (Cat. No. A5598)
- Anti-c-Myc-Alkaline Phosphatase Conjugate (Cat. No. A5963)
- Protease and phosphatase inhibitor cocktails (Cat. Nos. P2714, P8465, P8340, P8215, P9599, P2850, P5726, P0044, MSSAFE, P0001, PIC0002, PIC0004, PIC0005, PIC0006, PPC2020)
- BCA protein assay kits: Standard (Cat. No. BCA1) and QuantiPro™ (Cat. No. QPBCA)